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ORIGINAL ARTICLE

Gustatory Stimulations and Their Capacity Influence Buffering of the Saliva

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Abstract

Objective: To evaluate the influence of gustatory stimuli on the buffering capacity of saliva. **Material and Methods:** The buccal ph of 18 male volunteers aged 18-35 years was measured after a mouthwash with 20 ml of water as a control, and in individual disposable cups they collected the saliva for two minutes. Then, each of chewed bubble gum with sugar for two minutes, discarding the gum and made new collection of saliva, for two minutes in other disposable cups individualized. After collection, each volunteer was again subject to regular brushing with toothpaste and waited another ten minutes. The same procedure was repeated with all other substances. Salivary buffer capacity was determined by Ericsson technique. Data were submitted to analysis of variance and the means were compared by the Scott-Knott grouping test and Mann-Whitney test at 5% probability. Estimates of Pearson correlations were calculated in order to determine possible associations between the variables. **Results:** It was not found statistically significant differences between the initial pH variation and after eating food ($p>0.05$), or between gustatory stimulation and variation of salivary buffer capacity ($p>0.05$). **Conclusion:** There is no influence of gustatory stimulus aroma and flavor on the variation of salivary buffer capacity.

Keywords: Food; Taste; Hydrogen-Ion Concentration.

Introduction

Saliva is an important fluid in the body secreted by three pairs of larger salivary glands; the parotid, submandibular and sublingual. These glands secrete an average of 1.500 ml of saliva per day [1]. The salivary composition consists of several substances such as: lingual lipase, alpha salivary amylase and mucins, which aid in digestion, lubricate the food and protect oral mucosa. Immune globulin A (IgA) is an antibody that helps fight against viral and bacterial infections, is also present in saliva. Saliva also has other benefits such as facilitating swallowing, keeping mouth moist, dissolving molecules that stimulate buds, and the buffers in the saliva help to balance oral pH, with 7.0 being the normal pH in a secretion salivary at rest [2].

The gustatory sense is considered a chemical sense, because of its receptors that are caused by chemical stimulants present in food. Together with the olfactory sense, gustatory sense works on the perception of flavours. Each food activates a different combination of basic flavours. Many of these foods have a distinct taste as their taste and aroma. There are other sensory peculiarities that contribute to taste, such as texture and temperature of food [3].

The gustatory apparatus has as one of its functions, to provide stimuli to the salivary glands of the mouth. When the food is ingested, the gustatory stimulus, acting through reflexes transmitted in the brain, helps determine the amount of salivary secretion [4].

Reduced salivary flow is usually associated with a low buffering capacity, which can cause infections of the oral mucosa and periodontal disease. The salivary buffering capacity (SBC) is an important factor for dental caries resistance. A buffered system resists changes in pH occurring with the formation of acidic and basic ions, for example, by the fermentation of sugars, thus influencing the appearance of cavities. The most important salivary buffers are the carbonic acid/bicarbonate system and the phosphate system [2].

Dental caries is a disease with a higher prevalence in people with a low SBC, being a disease that begins before the development of the clinically detectable lesion. It affects all age groups and social classes, and even though there is a decline in disease rates at the end of the 20th century, a major public health problem is still considered by the World Health Organization [5].

The emergence of dental caries comes from a set of factors that interact with the tooth surface, such as dietary carbohydrates, saliva and plaque microorganisms. However, not all individuals have a similar propensity for the development of dental caries. The individual variability of caries risk is mainly related to the diet consumed and to the number of carcinogenic microorganisms present in bacterial plaque and saliva [6].

The acidity or sugar of various foods has little influence on salivary buffer capacity and gustatory stimuli provided by the food, whether pleasant or not, increase the salivary buffer capacity, and the pleasant taste (to the one who ingests the food) is a potentiate of buffering capacity and, lack of gustatory stimulus, characterized when people are indifferent or when they do not taste, as in the case of water is responsible for the worst buffer capacity [7].

There are not many reports in the literature correlating the buffering capacity of saliva to gustatory stimuli, and it is important to analyze whether or not the food interferes with salivary buffer capacity [8,9]. Within this context the aim of this study was to verify if the taste stimuli provided by the food interfere in the salivary buffer capacity (SBC) and the salivary pH.

Material and Methods

Study Design

This is an in vitro experimental study, developed between August and December 2014, in the multidisciplinary research laboratory at University Centre Cesmac.

Sample

For the accomplishment of the study were selected 18 people of the masculine gender with age between 18 and 35 years. The statistical parameters regarding the choice of volunteers were non-probabilistic, for convenience. All volunteers in the study had all healthy teeth.

Data Collection

Participants received a sensory evaluation sheet with an acceptance test, which evaluates how much they like or dislike a particular product. This test has a scale of 0 to 9, between extremely disliked and extremely enjoyed, and all participants had to score as to the taste and aroma of certain foods [10].

All participants were instructed to sit comfortably and wait five minutes before the start of the collections. Each participant made a mouthwash with 20 mL of water as a control and in individual disposable cups collected the saliva for two minutes. Then, each volunteer chewed a sugar-sweetened chewing gum for two minutes, then collecting saliva for another two minutes in other individualized disposable cups.

After the collection, each volunteer again brushed with toothpaste and waited another ten minutes, this being the method used for the standardization of the test and also for the buccal pH to stabilize after that waiting period [11].

The same procedure was repeated with all other substances: unsweetened chewing gum; chocolate with and without sugar; soda cola with and without sugar; olive oil; pure milk; natural fruit juice; fruit juice sweetened, noting that the products tasted were standardized, in relation to the brand, type of product, quantity and flavour.

In parallel to the consumption of each substance, the volunteers answered a questionnaire on aroma and taste, assigning the numerical values of the hedonic scale [7]: (9) like extremely; (8) like very much; (7) like moderately; (6) like slightly; (5) neither like nor dislike; (4) dislike slightly; (3) dislike moderately; (2) dislike very much and (1) dislike extremely.

At the end of the experiment, a water-based mouthwash was performed as final control, and a saliva sample was collected from each volunteer. Each sample was subjected to a pH measurement with triplicate colorimetric strips (MQuant®, Merck KGaA, Darmstadt, Germany) [12].

For the determination of SBC, the saliva/acid ratio was increased [13]. From each saliva sample, 1ml was pipette into the test tube containing 3ml hydrochloric acid (0.005M). The tube was then sealed with a rubber stopper and shaken for one minute on a mechanical tube shaker. Subsequently, the tubes were left on the shelf for ten minutes unopened in order to eliminate the carbon dioxide formed in the mechanical agitation. Only then the pH of the salivary sample was measured, and the SBC recorded as optimal when it had pH ≥ 5.6, regulate with pH between 4.5-5.5 and poor when pH ≤ 4.5 [14].

Statistical Analysis

The data were submitted to analysis of variance and the means were compared by the Scott-Knott group test at 5% probability. The Mann-Whitney test at 5% probability was applied to evaluate the hypothesis of equality between the initial SBC and the SBC after the food consumption and to detect if there is a difference between the initial SBC and the final SBC. This same test was also applied to evaluate the hypothesis of equality between initial pH of saliva and pH after food intake. In order to determine possible influences among the variables, estimates of Pearson correlations were calculated. All analyzes were performed using the Genes® software [15].

Ethical aspects

This study was approved by the Committee of Ethics in Education and Research of University Centre Cesmac (Protocol No. 741.227). After explaining the nature of the research, the volunteers signed the informed consent form.

Results

The evaluation of SBC in the initial and final (water) control of the experiment indicated that there were no differences between these variables, thus demonstrating reliability in the experiment. It was observed that there is a difference between initial salivary buffer capacity and SBC after food consumption ($p<0.05$).

The mean values of initial SBC, SBC after food consumption, SBC variation, aroma and flavour of the different foods studied are shown in Table 1. There was a significant difference between foods, aroma and flavour variables. In general, it was observed that the foods that presented the highest averages were: sugar chewing gum, diet chewing gum, chocolate with sugar, chocolate without sugar. The results obtained show that there was no statistical difference between the foods in relation to the SBC variation.

Table 1. Salivary buffering capacity means, aroma and flavour in relation to the sensorial analysis.

Foods	iSBC*	foodSBC**	SBCVar***	Aroma	Flavor
Sugar-Sweetened Chewing Gum	5.39[a]	5.94[a]	0.56[a]	7.28[a]	7.72[b]
Unsweetened Chewing Gum	5.42[a]	6.14[a]	0.72[a]	6.72[b]	8.11[a]
Chocolate with Sugar	5.42[a]	6.14[a]	0.72[a]	8.00[a]	8.56[a]
Chocolate without Sugar	5.44[a]	6.03[a]	0.58[a]	7.44[a]	7.17[b]

Soda Cola	5.42[a]	5.86[a]	0.44[a]	6.61[b]	7.50[b]
Soda Cola without Sugar	5.42[a]	5.75[a]	0.33[a]	6.22[b]	5.44[c]
Pure Milk	5.39[a]	5.97[a]	0.58[a]	5.78[b]	3.67[d]
Olive Oil	5.39[a]	6.14[a]	0.75[a]	6.67[b]	6.94[b]
Natural Fruit Juice	5.42[a]	5.92[a]	0.50[a]	5.89[b]	4.83[c]
Fruit Juice Sweetened	5.42[a]	5.97[a]	0.57[a]	6.28[b]	6.05[c]

*iSBC= initial SBC; **foodSBC= SBC after food intake; ***SBCVar= SBC variation. Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott grouping test at the 5% probability level.

Estimates of correlations between the variables demonstrated that there was a strong and positive correlation ($p < 0.01$) between SBC after food consumption and SBC variation ($r = 0.9875$), between flavour and taste ($r = 0.8528$) and between initial SBC and pH variation ($r = -0.7276$). In the latter case it is important to note that the negative sign in Pearson correlation coefficient (r) indicates that these two variables are inversely proportional. No correlation was found between pH and variation in SBC. No correlation was found between the initial salivary pH and salivary pH after food intake, ie, food consumption did not influence pH. It was possible to observe that the gustatory stimulus (aroma and flavour parameters) did not influence the SBC variation ($r = 0.4398$ and $r = 0.4481$, respectively) and pH variation ($r = 0.2203$ and $r = 0.973$, respectively) (Table 2).

Tabel 2. Estimates of Pearson correlations (r) among variables related to sensory analysis.

Food	pH	iSBC*	foodSBC**	SBCVar***	Aroma	Flavor	pHV****
pH	-	-0.1405	0.2066	0.2364	0.2203	0.4973	0.2426
iSBC	-	-	-0.0945	-0.2397	0.2321	0.2314	-0.7276¥
foodSBC	-	-	-	0.9875¥	0.4896	0.4891	0.2171
SBCVar	-	-	-	-	0.4398	0.4481	0.3233
Aroma	-	-	-	-	-	0.8528¥	0.2829
pHV	-	-	-	-	-	0.2048	-

*iSBC= initial SBC; **foodSBC= SBC after food intake; ***SBCVar= SBC variation; ****pH= pH variation; ¥: Significant at 1% by the t test.

Regarding the variation between initial pH and pH after food intake, it was found that there was a significant difference ($p<0.05$) in chewing sugar and sugar-free chewing gum products. The other foods did not show differences between the pH variations (Table 3).

Table 3. Initial pH and pH after food intake means.

Food	Initial pH	Food pH
Sugar-Sweetened Chewing Gum	7.3889*	7.8056*
Unsweetened Chewing Gum	7.3889**	7.9167**
Chocolate with Sugar	7.3889	7.5278
Chocolate without Sugar	7.3889	7.4444
Soda Cola	7.3889	7.5833
Soda Cola without Sugar	7.3889	7.4722
Pure Milk	7.3889	7.5000
Olive Oil	7.3889	7.4167
Natural Fruit Juice	7.3889	7.4444
Fruit Juice Sweetened	7.3889	7.5278

* and ** They differ statistically from each other by the Mann-Whitney test at the 5% probability.

Discussion

The similarity between the conditions found in initial and final controls suggests that there was no interference from plaque removal performed by the various brushings (ten minutes waiting to eliminate intervention between foods) or from the action of toothpaste components (such as fluoride and abrasive based baking soda) on the results of the experiment [11].

The influence of the SBC gustatory stimulus obtained in the present study differs from the results found in other studies that suggest that there is interference between gustatory stimuli for different foods, describing that food taste varied in the SBC [7]. It is important to point out that further research is needed to ascertain the role of diet in SBC, to salivate and to relate them to the onset and evolution of dental caries. It is also necessary that the sample is formed only by male volunteers, since the hormonal variation can influence the gustatory stimuli of the woman [7].

These indications corroborate with the present study, which was performed with a larger number of volunteers, all of them male. These constraints may have led to changes in the results obtained when compared with other studies [7].

The non-influence of juice consumption on pH variation differs from results found in other studies, in which a reduction of pH was observed [16]. After 5 minutes of juice ingestion, 3 children in the study had pH below that value, and the other 28 children had pH above that value. According to the same author the elevation of pH may have occurred because the stimulation of the salivary flow produced by the juice, which has an extremely low pH, or by sugars.

It is noteworthy that articles described, did not develop the same methodology, of the present study, diverging volunteer's ages, approach and procedures developed. This corroborates the differences found. Several papers have been published related to the determination of SBC, salivary flow, dental erosion, gustatory stimuli correlated to salivary flow and pH [17].

In addition to these approaches mentioned above, there are several articles related to gustatory stimuli with another theme among them, taste perception in patients with stroke [16]; evaluation of taste perception before and after physical exercise [19]; effect of the association of flavour and music on the mood of children and the influence of the aroma of the food [20,21]. However, there are few reports on the influence of salivary buffer capacity evaluation on gustatory stimuli, which certainly hinders the discussion of the present research and at the same time evidences its importance.

Conclusion

No correlation was found between pH and buffering capacity variation, evidencing that there is no influence between them. There was no correlation between initial pH of saliva and the pH of saliva after ingestion of the food, that is, food consumption did not influence the pH. It was observed that the taste stimulus did not influence the salivary buffer capacity variation and the pH variation, only the chewing gum and sugar-free chewing gum products promoted a significant difference. It is worth noting that other studies are necessary, relating gustatory stimuli and salivary buffer capacity,

preferably with a larger number of volunteers, which would allow the individualized analysis of the influence of the taste stimulus resulting from a specific food in relation to salivary buffer capacity.

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Conflict of Interest: The authors declare no conflicts of interest.

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